

[54] **COMPOUND NANAOMYCIN A AND DERIVATIVES THEREOF AND A PROCESS FOR PRODUCING THE SAME**

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# **Related U.S. Application Data**

[60] Division of Ser. No. 858,215, Dec. 7, 1977, Pat. No. 4,196,266, which is a continuation of Ser. No. 719,744, Sep. 2, 1976, abandoned, which is a continuation-in-part of Ser. No. 558,563, Mar. 14, 1975, abandoned.

# **Foreign Application Priority Data**

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[52] U.S. Cl. .... **260/345.2**

[58] Field of Search ..... 424/122, 283; 260/345.2

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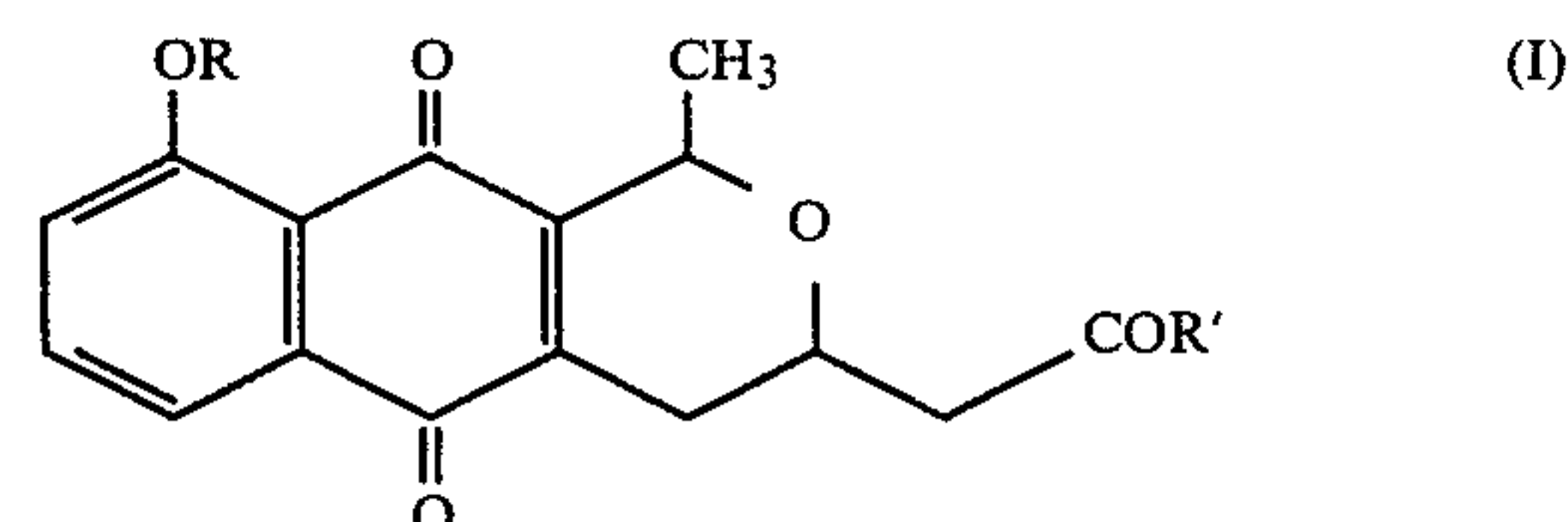
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# **[57] ABSTRACT**

The present invention relates to new compound nanaomycin A and derivatives thereof represented by general formula:



in which

- (a) R is H and R' is OH (nanaomycin A),
- (b) R is H and R' is NH<sub>2</sub> (nanaomycin C),
- (c) R is COCH<sub>3</sub> and R' is OH (acetylnanaomycin A), and
- (d) R is H and R' is OCH<sub>3</sub> (nanaomycin A methyl ester).

Nanaomycin A is a new compound of quinone type and its acute toxicity (LD<sub>50</sub>, intra-peritrial injection) in mice is 28.2 mg/Kg. Nanaomycin A and derivatives thereof are active on Gram-positive bacteria, trichophyton and mycoplasma and are useful as a medicament for humans and animals. Nanaomycins A and C are produced by culturing a nanaomycin-producing strain belonging to the genus *Streptomyces* aerobically in a medium to accumulate nanaomycins A and C in the cultured broths. The derivatives acetylnanaomycin A and nanaomycin A methyl ester have similar properties to those of nanaomycin A.

**5 Claims, 10 Drawing Figures**

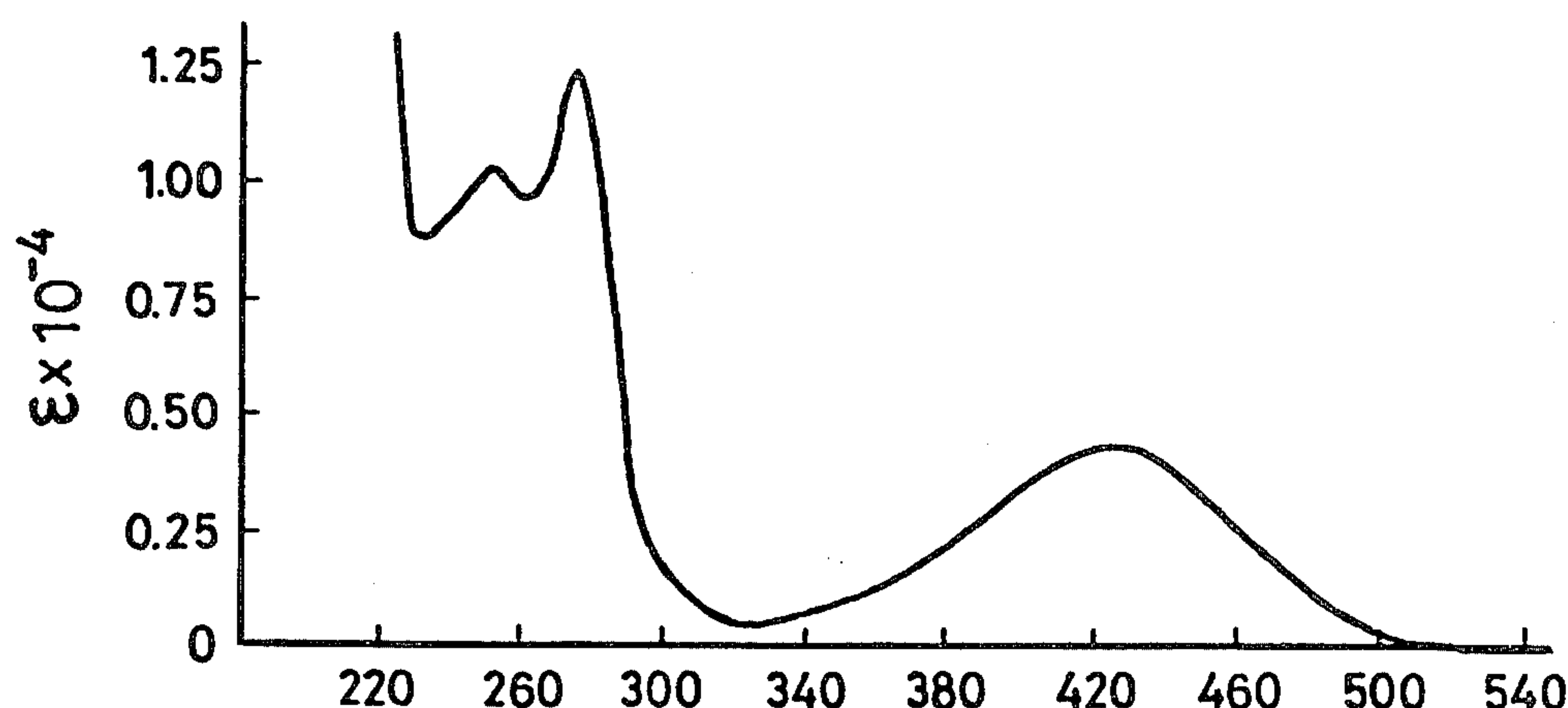


FIG. 1

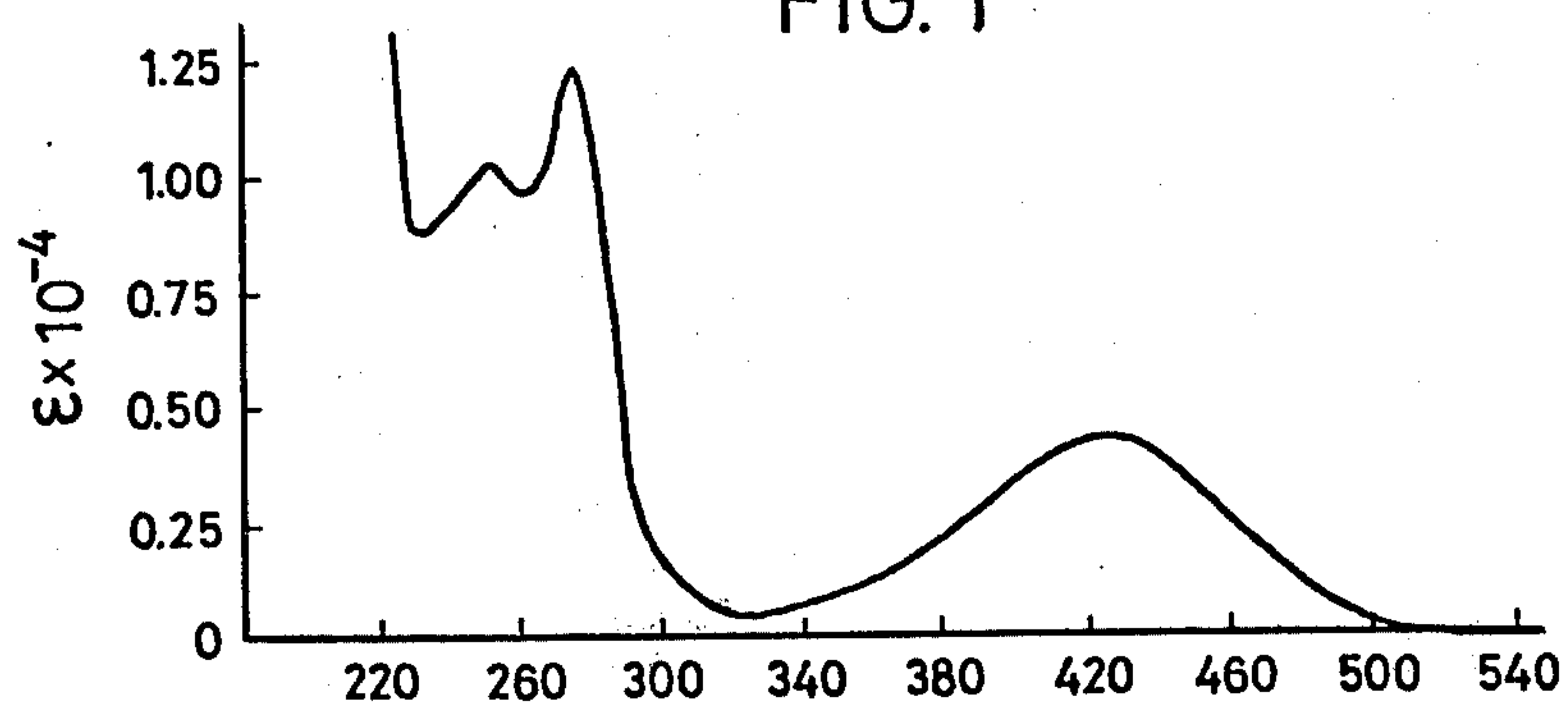


FIG. 2

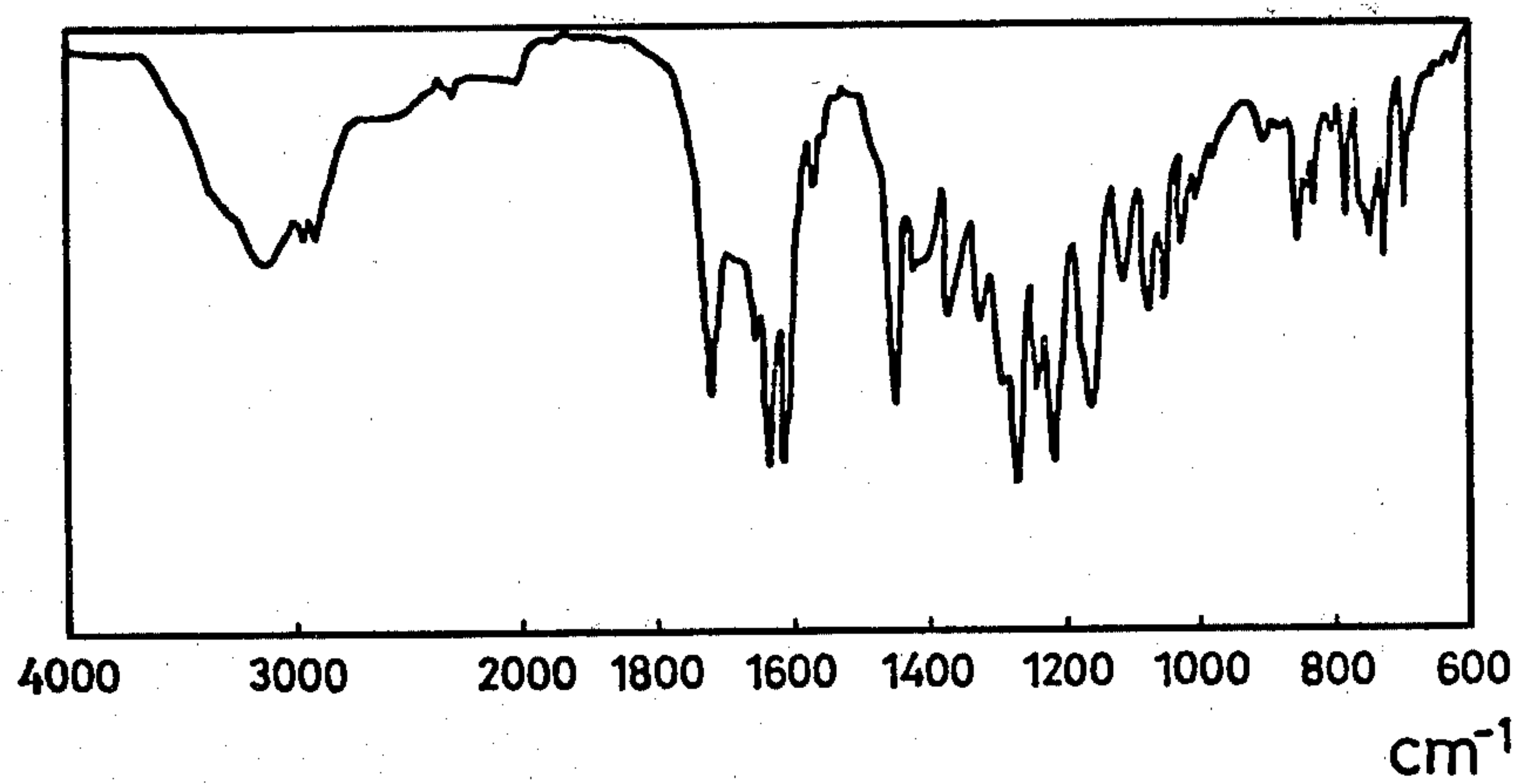
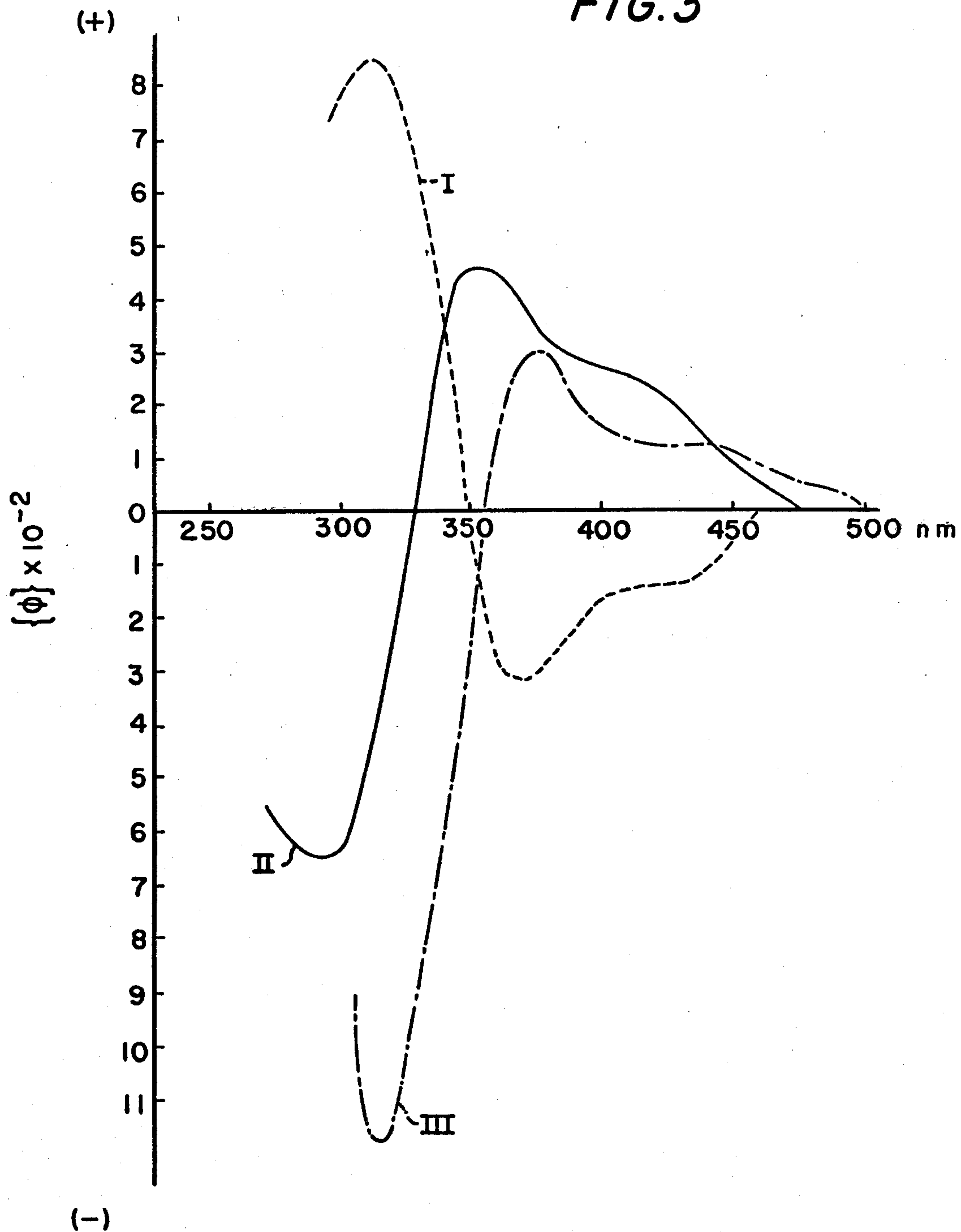


FIG. 3



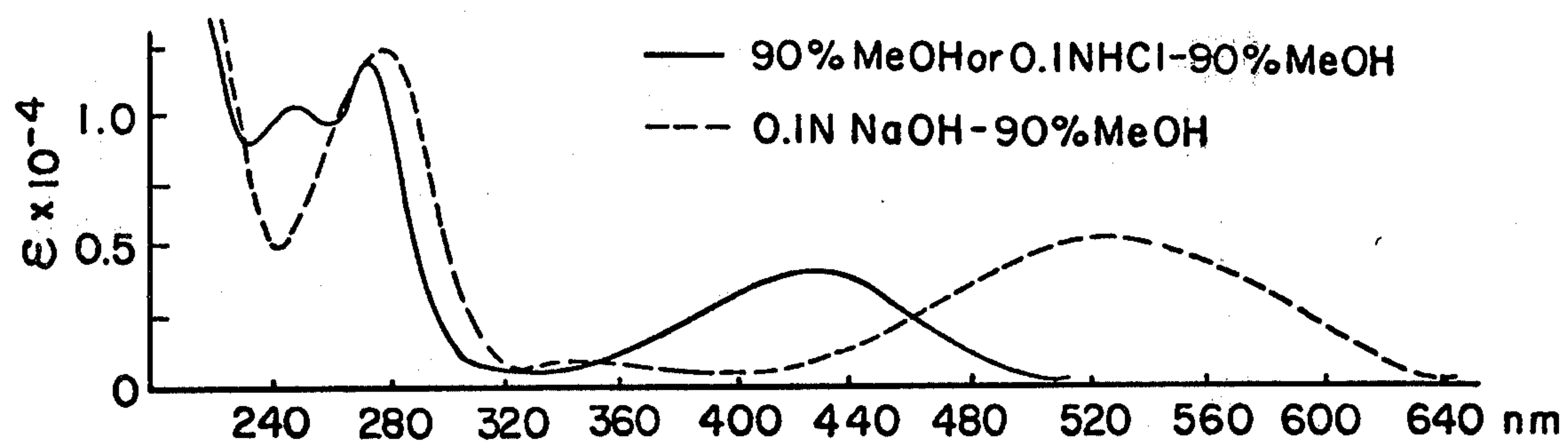
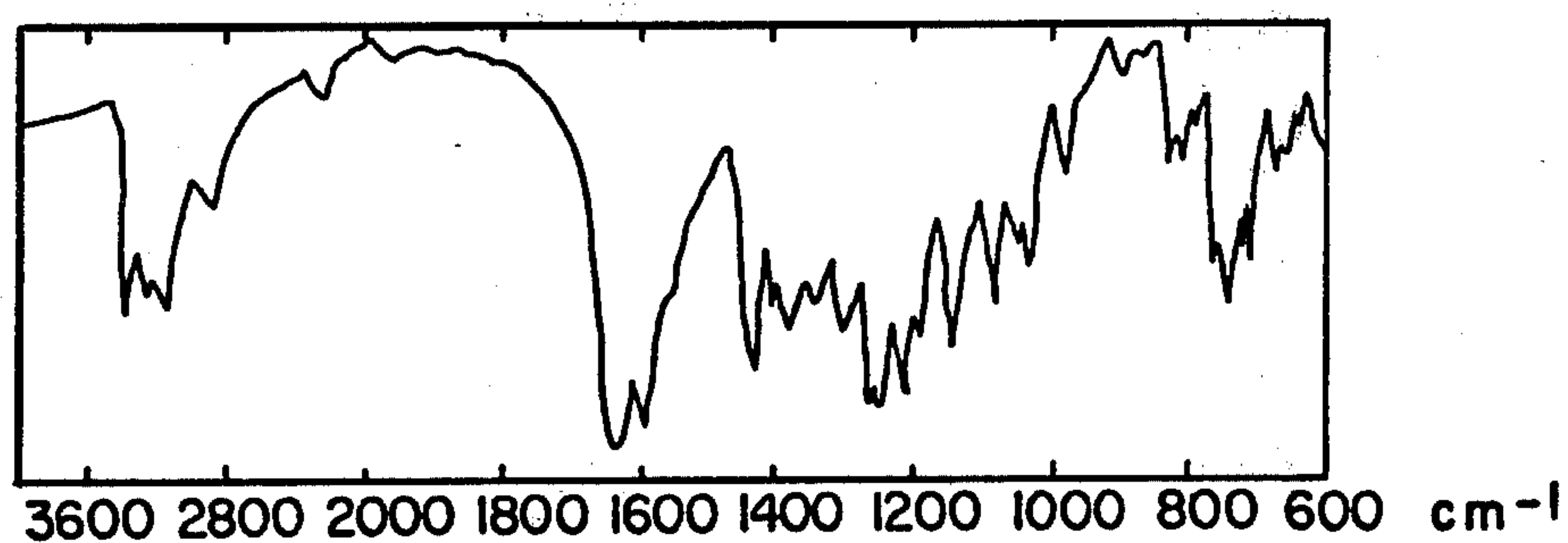
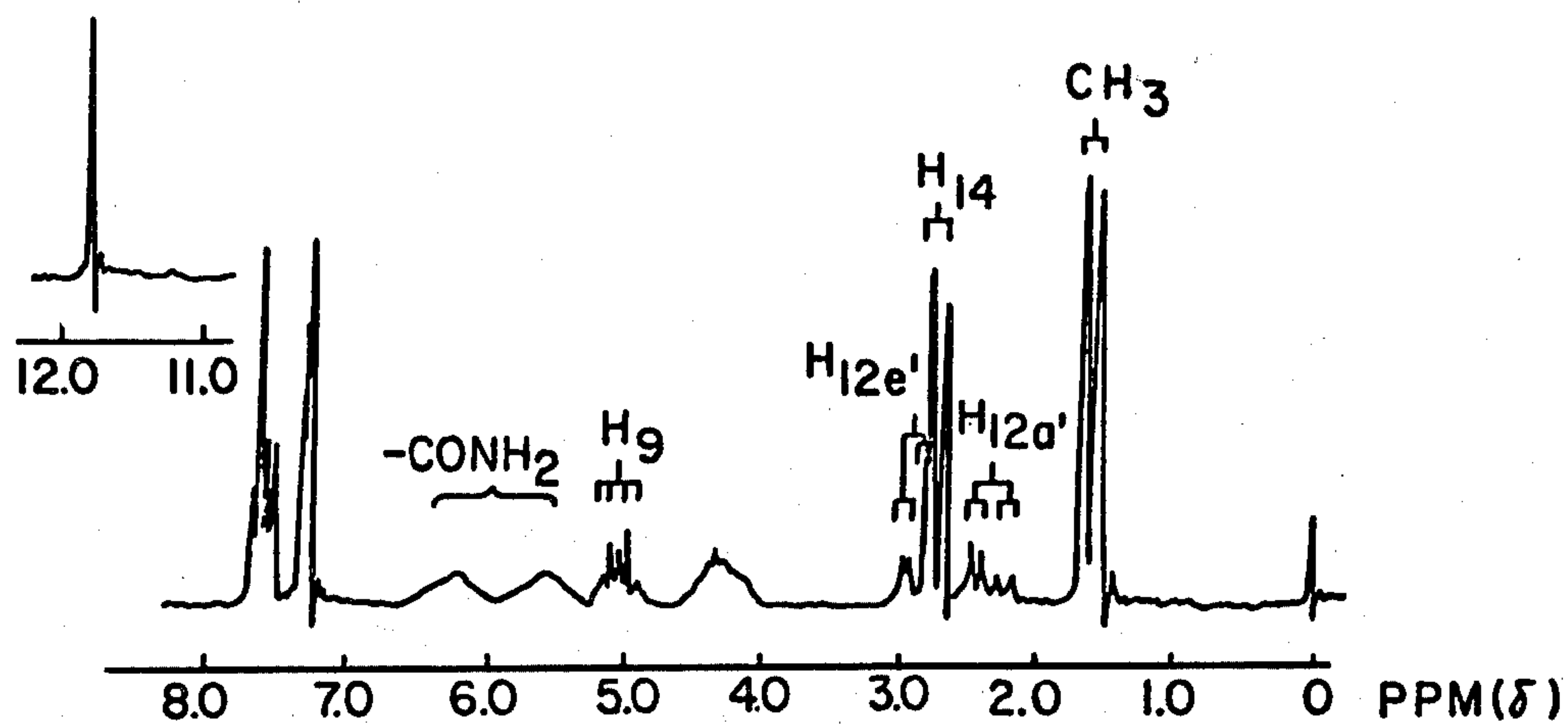
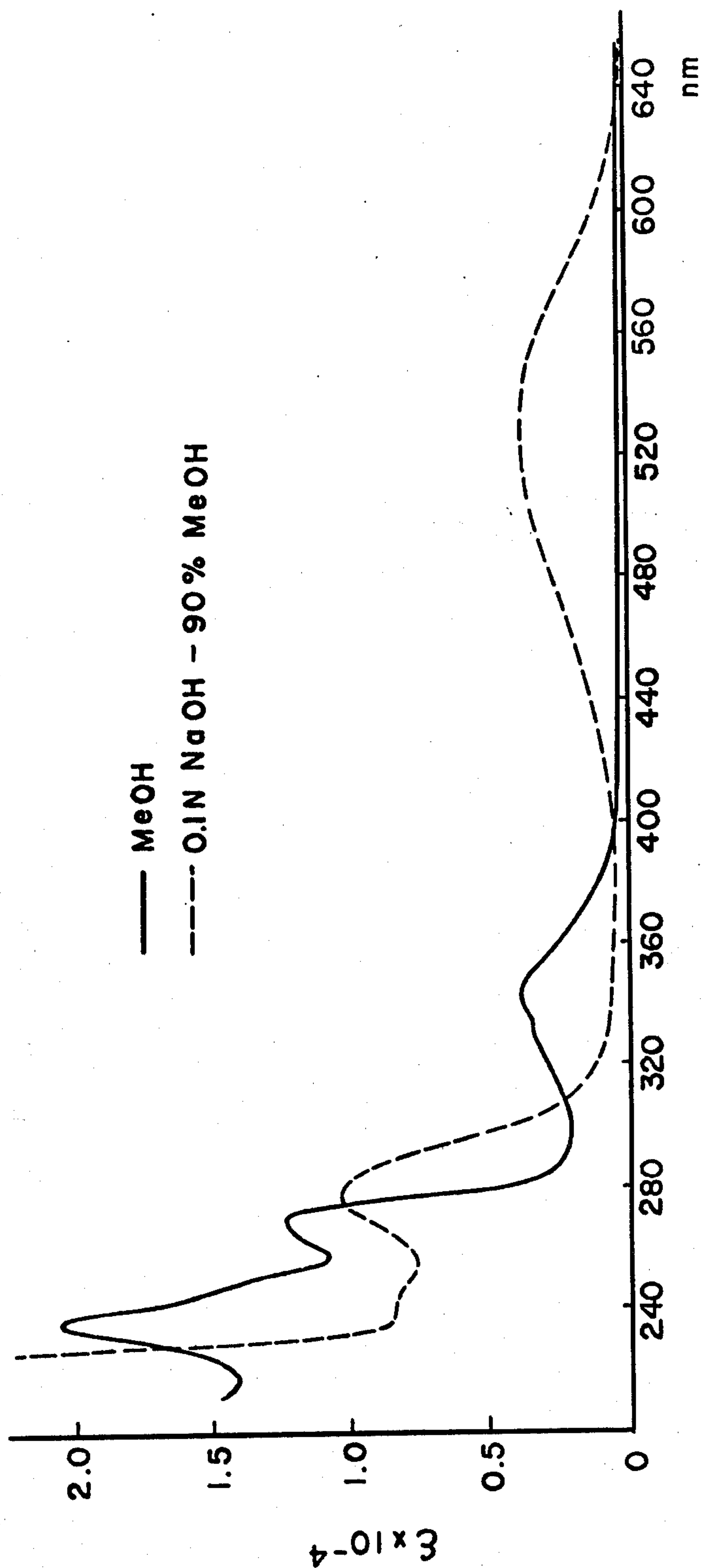
**FIG. 4****FIG. 5****FIG. 6**

FIG. 7



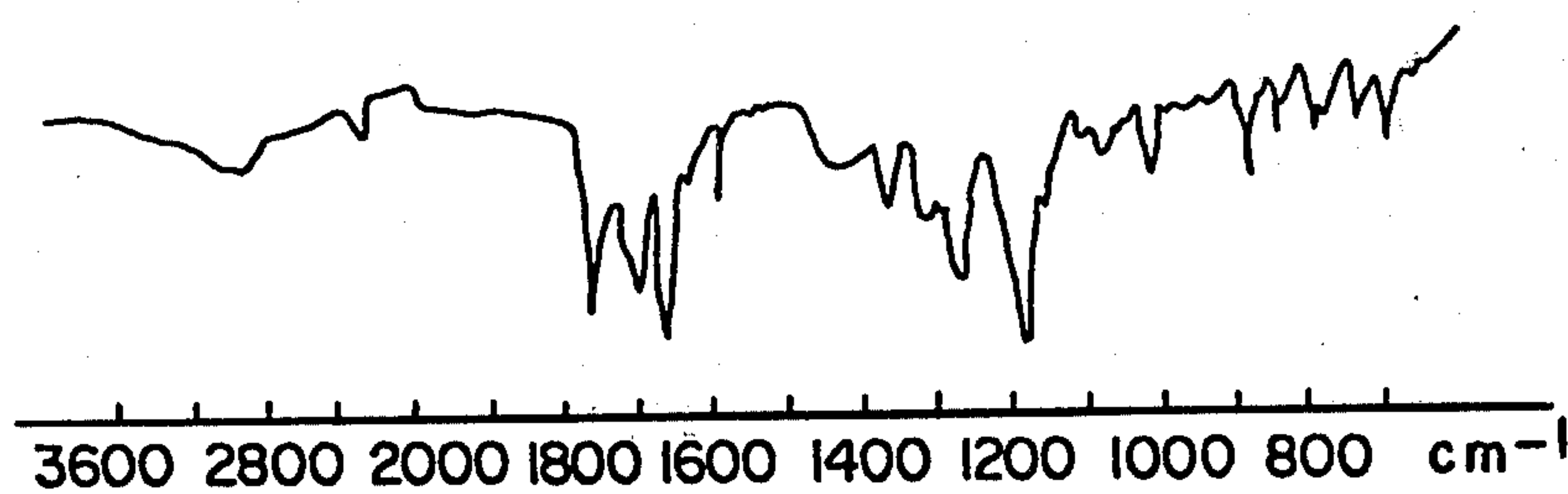
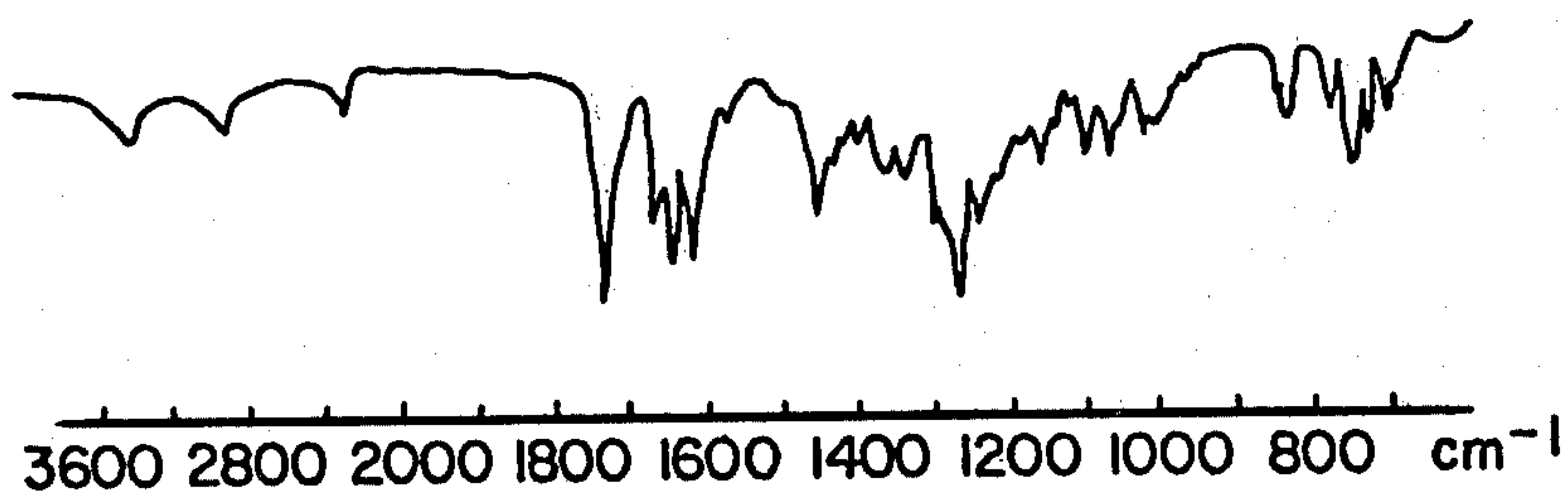
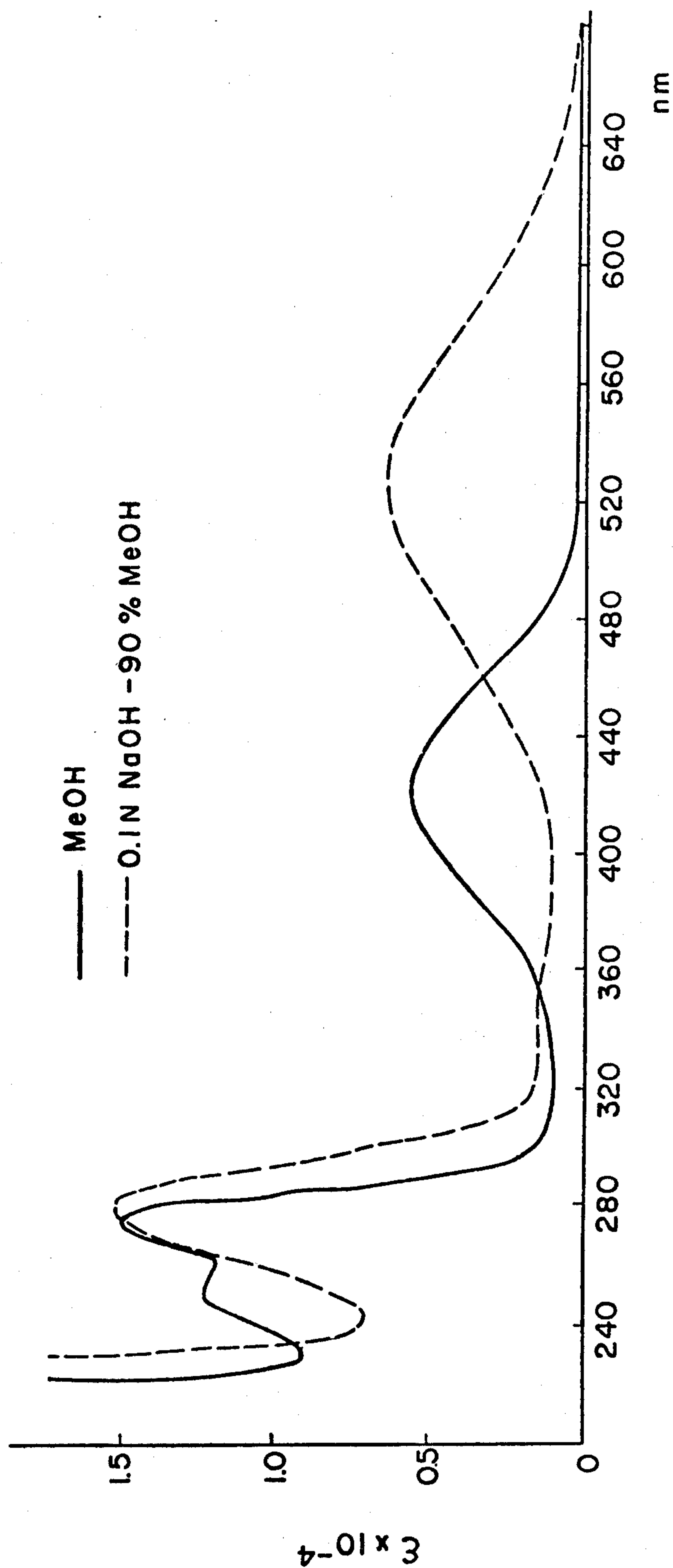
**FIG. 8****FIG. 10**



FIG. 9



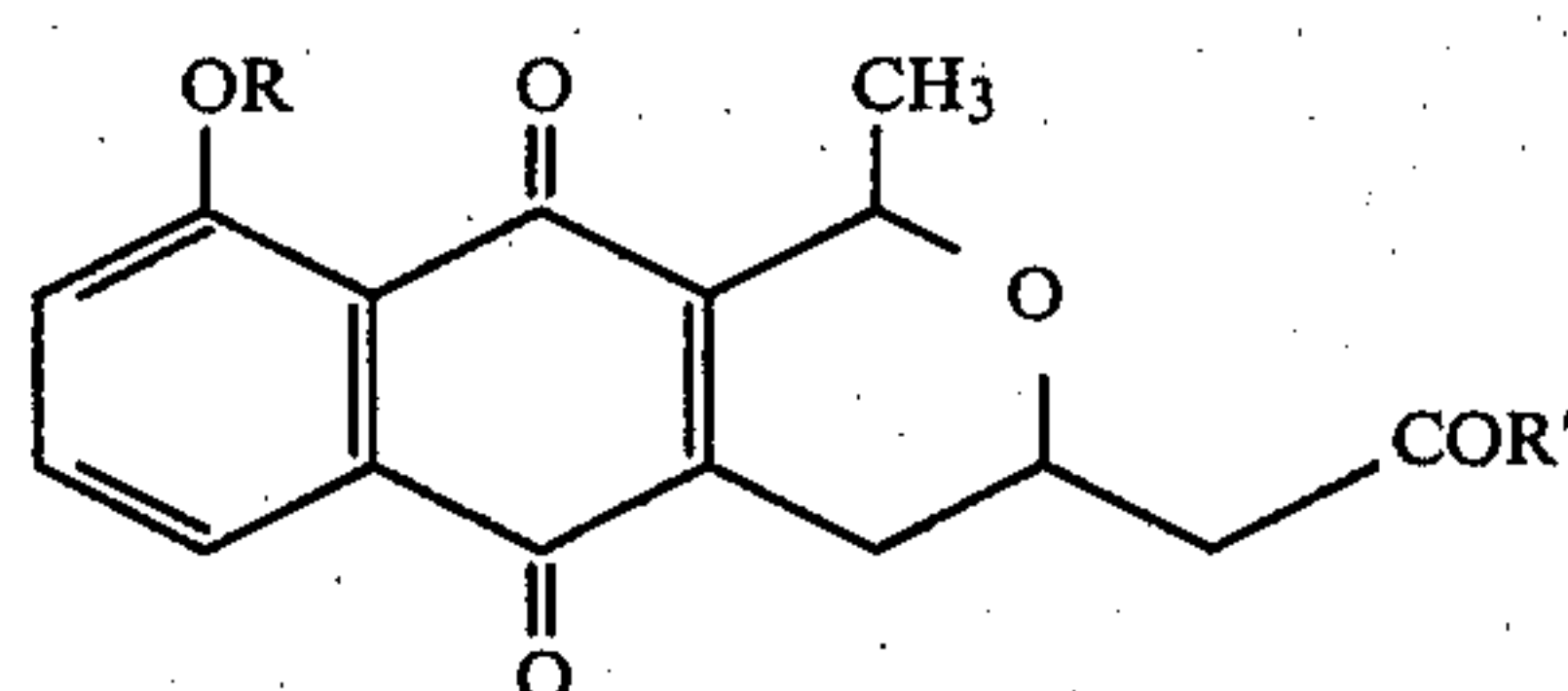
# COMPOUND NANAOMYCIN A AND DERIVATIVES THEREOF AND A PROCESS FOR PRODUCING THE SAME

## RELATED APPLICATION

"The present application is a divisional of U.S. Ser. No. 858,215 filed Dec. 7, 1977 which is now U.S. Pat. No. 4,196,266 which is a continuation of Ser. No. 719,744, filed Sept. 2, 1976 (now abandoned) which is a continuation in part of Ser. No. 558,563, filed Mar. 14, 1975 (now abandoned,) all for the same inventions".

## SUMMARY OF THE INVENTION

The present invention relates to new compound designated as nanaomycin A which is also designated as OS-3966-A or Rosanomycin A. and derivatives thereof represented by general formula:



in which

- (a) R is H and R' is OH,
- (b) R is H and R' is NH<sub>2</sub>,
- (c) R is COCH<sub>3</sub> and R' is OH, and
- (d) R is H and R' is OCH<sub>3</sub>.

In this specification, the compounds of general formula (I)—(a), —(b), —(c) and —(d) are designated as nanaomycin A, nanaomycin C, acetylnanaomycin A and nanaomycin A methyl ester, respectively. The above-mentioned formula has been determined by various experiments including nuclear magnetic resonance spectra, elementary analysis, mass-spectra and the like.

## DRAWINGS

- FIG. 1—UV absorption spectrum of nanaomycin A
- FIG. 2—IR absorption spectrum of nanaomycin A
- FIG. 3—ORD curves of (I) nanaomycin A, (II) kalamycin and (III) deoxyfrenolicin
- FIG. 4—UV absorption spectrum of nanaomycin C
- FIG. 5—IR absorption spectrum of nanaomycin C
- FIG. 6—NMR spectrum of nanaomycin C
- FIG. 7—UV absorption spectrum of acetylnanaomycin A
- FIG. 8—IR absorption spectrum of acetylnanaomycin A
- FIG. 9—UV absorption spectrum of nanaomycin A methyl ester
- FIG. 10—IR absorption spectrum of nanaomycin A methyl ester

Nanaomycin A and derivatives thereof according to the present invention are active upon mycoplasma, Gram-positive bacteria and trichophyton, and the acute toxicity (LD<sub>50</sub>, intra-penetril injection) in mice of nanaomycin A is 28.2 mg/Kg. These compounds have an excellent therapeutic effect on infectious diseases caused by a parasite of Gram-positive bacteria, trichophyton or mycoplasma. It has now been found that the properties of nanaomycin A and derivatives thereof according to the present invention are different from

those of known antibiotics such as deoxyfrenolicin and ethylkalamycin (ethylkalamycin).

Nanaomycin A is in the form of crystals of yellow needles and has the following physical and chemical characteristics:

1. Elementary analysis:  
Found: C: 63.35%; H: 4.47%; N: 0%,  
Calculated (as C<sub>16</sub>H<sub>14</sub>O<sub>6</sub>): C: 63.57%; H: 4.66%; N: 0%.
2. Molecular weight: m/e determined by mass spectrum is 302.084 and the theoretical value of m/e for C<sub>16</sub>H<sub>14</sub>O<sub>6</sub> is 302.079.
3. Melting point: 178°–180° C.
4. Specific rotation:  $[\alpha]_D^{26} -27.5^\circ$  (C=1.0 in methanol)
5. Ultraviolet absorption spectrum (FIG. 1):  $\lambda_{max-MeOH}$  nm( $\epsilon$ ): 250 (0.985×10<sup>4</sup>), 274 (1.22×10<sup>4</sup>), 423 (0.404×10<sup>4</sup>)
6. Infrared absorption spectrum (FIG. 2):  
Relatively strong absorptions at 3150, 2960, 2910, 1725, 1640, 1610, 1450, 1370, 1320, 1270, 1220, 1160 cm<sup>-1</sup> when measured by KBr method.
7. Solubility:  
Easily soluble in methanol, ethanol, ethylacetate, chloroform, acetone and ether. Insoluble in n-hexane, petroleum ether and water.
8. Color reaction:  
Positive in the reactions with ferric chloride and reduction catalyst [Feigl, N., Anal. Chem., 28, 397 (1956)]. Negative in ninhydrin reaction, Sakaguchi reaction, Ehrlich reaction, Fehling reaction and Molish reaction.

From the characteristics stated above, it has been found that nanaomycin A is a new quinone type compound. Nanaomycin A is also distinguishable from kalamycin (kalamycin) and deoxyfrenolicin owing to the difference of the specific rotation and optical rotatory dispersion curve (ORD curve) shown in FIG. 3.

The antimicrobial spectra of nanaomycin A is shown in Table 1.

TABLE 1

Test Organisms	Medium	MIC (μg/ml)
<i>Bacillus subtilis</i> PCI 219	N	7.8
<i>Staphylococcus aureus</i> FDA 209P	N	3.9
<i>Staphylococcus aureus</i> FDA 209P(JC-1)	N	2.0
<i>Sarcina lutea</i> PCI 1001	N	2.0
<i>Mycobacterium smegmatis</i>	N	62.5
<i>Escherichia coli</i> NIHJ	N	31.3
<i>Escherichia coli</i> NIHJ(JC-2)	N	250
<i>Klebsiella pneumoniae</i> PCI 602	N	31.3
<i>Salmonella typhimurium</i>	N	62.5
<i>Shigella flexneri</i>	N	31.3
<i>Xanthomonas oryzae</i> N-5824	N	62.5
<i>Pseudomonas aeruginosa</i>	N	500
<i>Candida albicans</i>	P	31.2
<i>Saccharomyces sake</i>	P	31.2
<i>Aspergillus niger</i> ATCC 6275	P	62.5
<i>Aspergillus fumigatus</i> IAM 2612	P	12.5
<i>Piricularia oryzae</i>	P	7.8
<i>Microsporium gypseum</i> 704	P	0.8
<i>Trichophyton asteroides</i>	P	1.6
<i>Trichophyton ferrugineum</i>	P	1.6
<i>Trichophyton interdigitale</i>	P	1.6
<i>Trichophyton mentagrophytes</i>	P	0.8
<i>Trichophyton pedis</i> 804	P	0.2
<i>Trichophyton purpureum</i>	P	3.1
<i>Trichophyton roseum</i>	P	0.4
<i>Trichophyton rubrum</i>	P	<0.1
<i>Trichophyton schoenleini</i>	P	0.2
<i>Trichophyton violaceum</i>	P	0.4
<i>Mycoplasma gallisepticum</i> KP-13	H	<0.013
	E	0.05



TABLE 1-continued

Test Organisms	Medium	MIC (μg/ml)
<i>Mycoplasma gallisepticum</i> S-6	H	<0.013
	E	0.10
<i>Mycoplasma gallisepticum</i> 333P (Spiramycin resistant)	H	<0.013
	E	<0.013
<i>Mycoplasma gallinarum</i>	H	1.56
<i>Mycoplasma iners</i>	H	3.12
<i>Mycoplasma pneumoniae</i>	E	0.013
<i>Acholeplasma laidlawii</i> (A) PG8	H	>25
	E	>25
<i>Acholeplasma laidlawii</i> (B) Bml	H	25
	E	>25

Note:  
Medium N - nutrient agar (pH 7.0, 2 days, 37° C.)  
P - potato agar (pH 6.4, 4 days, 27° C.)  
H - Hokken PPLO agar (pH 7.8, 8 days, 37° C.)  
E - Eiken PPLO agar (pH 7.8, 8 days, 37° C.)  
MIC - Minimal inhibitory concentration

As apparent from Table 1, nanaomycin A exhibits a strong activity against Gram-positive bacteria. For example, the growth of *Staphylococcus aureus* is inhibited at a concentration of 2.0 to 8.0 μg/ml and the growth of some fungi belonging to the genus *Trichophyton* is inhibited at a concentration of not more than 3.1 μg/ml. Nanaomycin A has also a high activity against mycoplasma and the growth of *Mycoplasma gallisepticum* is inhibited at a concentration of not more than 0.1 μg/ml. Furthermore, nanaomycin A exhibits a strong activity against a spiramycin-resistant *Mycoplasma gallisepticum*.

Nanaomycin A exhibits an excellent therapeutic effect on various infectious diseases of animals caused by a parasite of the genus *Trichophyton*. For example, when dermatomycosis caused by *Tricophyton metagorphytes* at the back of guinea pigs is treated with a solution of 0.01-1% nanaomycin A dissolved in propylene-glycolethanol (3:1 v/v) once daily for 8 days, an excellent therapeutic effect on erythema and scales is observed. Furthermore, nanaomycin A has a therapeutic effect on infectious diseases of animals caused by a parasite of the genus *Mycoplasma* and exhibits therapeutic effect on chronic respiratory disease of chickens caused by *Mycoplasma gallisepticum*.

When dermatomycosis of cattle caused by mass infection of *Trichophyton verrucosum* is treated with nanaomycin A, nanaomycin A is dissolved or suspended in a suitable carrier, for example, olive oil, and is coated on the affected part directly with or without removal of the scales. SP-Burton (a commercial product available from Rakuno-shinko K. K., Japan) which is considered as a most effective agent for dermatomycosis of animals is used as a control therapeutic agent. The therapeutic effects are observed for 4 weeks. The results obtained by once-coating of nanaomycin A and by twice-coating (the second coating is carried out a week after the first coating) are shown in Tables 2 and 3, respectively.

TABLE 2

Agent	Con. mg/ ml	Sam- ple	Result AFTER			
			1 week	2 weeks	3 weeks	4 weeks
I Nanaomycin A	0.1	1	++	++	+++	+++
Nanaomycin A	1	1	+++	+++	+++	+++
Nanaomycin A	10	1	+++	+++	+++	+++
SP-Burton	0.3	1	—	—	—	—
II Nanaomycin						

TABLE 2-continued

Agent	Con. mg/ ml	Sam- ple	Result AFTER			
			1 week	2 weeks	3 weeks	4 weeks
A	0.1	1	+++	+++	+++	+++
Nanaomycin A	1	1	+++	+++	+++	+++
Nanaomycin A	10	1	+++	+++	+++	+++
Sp-Burton	0.3	1	+++	+	—	—

TABLE 3

Agent	Con. mg/ ml	Sam- ple	Result AFTER			
			1 week	2 weeks	3 weeks	4 weeks
I Nanaomycin A	0.1	1	++	+++	+++	+++
Nanaomycin A	1	1	++	++	+++	+++
Nanaomycin A	10	1	+++	+++	+++	+++
SP-Burton	0.3	1	—	—	—	—
II Nanaomycin A	0.1	1	+++	+++	+++	+++
Nanaomycin A	1	1	+++	+++	+++	+++
Nanaomycin A	10	1	+++	+++	+++	+++
SP-Burton	0.3	1	+++	++	+	—

Note for Tables 2 and 3:  
I : direct coating on affected part  
II : coating after removing the scales of affected part  
Con. : concentration of agent  
+++ : complete removal of scales and recovered  
++ : a little scales remained  
+ : removal of a part of scales  
— : no removal of scale and no recovery

The anti-fungal activity and antimycoplasma activity of nanaomycin A are shown in Tables 4 and 5, respectively.

TABLE 4

Test Organisms	Antifungal Activity		
	Minimal Inhibitory Con. (μg/ml)		
	Nanaomycin A	Y	Z
<i>Candida albicans</i>	50	50	>100
<i>Saccharomyces sake</i>	12.5	6.3	>100
<i>Aspergillus fumigatus</i>	6.3	6.3	>100
<i>Aspergillus niger</i>	25	>100	>100
<i>Microsporium gypseum</i>	0.8	<0.2	>100
<i>Trichophyton asteroides</i>	0.8	<0.2	>100
<i>Trichophyton ferrugineum</i>	0.8	3.1	>100
<i>Trichophyton interdigitale</i>	1.6	0.4	>100
<i>Trichophyton rubrum</i>	<0.2	<0.2	>100
<i>Trichophyton schoenleini</i>	<0.2	<0.2	>100
<i>Trichophyton violaceum</i>	3.1	0.8	>100

Note:  
Minimal inhibitory concentration was assayed by agar dilution method (potato agar, pH 6.4, 27° C., 4 days).  
Y : deoxyfrenolicin  
Z : ethylkalafunginate (ethylkalamycinatate)

TABLE 5

Antibiotic	Antimycoplasma Activity	
	Concentration (μg/ml)	Inhibitory Zone (mm)
Nanaomycin A	10	20.4
	100	28.7
Deoxyfrenolicin	10	14.6
	100	21.8
Ethylkalafunginate	10	none



TABLE 5-continued

Antibiotic	Antimycoplasma Activity	
	Concentration ( $\mu\text{g}/\text{ml}$ )	Inhibitory Zone (mm)
	100	none

Note:

Inhibitory zone is assayed by paper disc method (Eiken PPLO agar, pH 7.8, 37° C., 1 day).

As shown in Table 5, the antimycoplasma activity of nanaomycin A is superior to those of deoxyfrenolicin and ethylkalafunginate.

Further study of nanaomycins has now led to the discovery of some nanaomycin derivatives, namely nanaomycin C, acetylnanaomycin A and nanaomycin A methyl ester, having similar properties to those of nanaomycin A. The derivatives of nanaomycin A according to the present invention have the following physical and chemical properties.

## (A) Nanaomycin C:

Nanaomycin C is neutral and in the form of crystals of orange needles.

## 1. Elementary analysis:

Found: C: 63.46%; H: 4.50% N: 4.89%;

Calculated (as  $\text{C}_{16}\text{H}_{15}\text{NO}_5$ ): C: 63.78%; H: 4.64%; N: 5.02%.

## 2. Molecular weight:

m/e determined by mass spectrum is 301.092 and the theoretical value of m/e for  $\text{C}_{16}\text{H}_{15}\text{NO}_5$  is 301.095.

## 3. Melting point: 222°–224° C. (decomposition)

4. Specific rotation:  $[\alpha]_D^{26} - 2^\circ$  (C=0.5 in dioxane)

## 5. Ultraviolet absorption spectrum (FIG. 4):

$\lambda_{\text{max}}^{\text{MeOH}}$  nm( $\epsilon$ ): 248 (10100), 274 (12400), 424 (4610)

## 6. Infrared absorption spectrum (FIG. 5):

Relatively strong absorptions at 3400, 3260–70, 3180, 2960, 2910, 1645, 1605, 1570, 1450, 1420, 1392, 1360, 1315, 1278, 1263, 1233, 1210, 1160 and 1103  $\text{cm}^{-1}$  when measured by KBr method.

## 7. Solubility:

Soluble in methanol, ethanol, ethylacetate, chloroform and acetone. Insoluble in water, n-hexane, and petroleum ether.

## 8. Color reaction:

Positive in the reactions with ferric chloride, 2,4-dinitrophenyl hydrazine and formaldehyde-0-dinitrobenzene. Negative in ninhydrin reaction, Ehrlich reaction and Sakaguchi reaction.

## 9. Rf value:

0.35 in silica gel thin-layer chromatography using chloroform-methanol (10:1 v/v).

## 10. Nuclear magnetic resonance (NMR) spectrum:

Shown in FIG. 6.

From the characteristics stated above, it has been found that nanaomycin C is a new compound similar to nanaomycin A and is an acid amide of nanaomycin A.

## (B) Acetylnanaomycin A:

Acetylnanaomycin A is in the form of crystals of pale yellow needles.

## 1. Elementary analysis:

Found: C: 62.89%; H: 4.73%; N: 0%;

Calculated (as  $\text{C}_{18}\text{H}_{16}\text{O}_7$ ): C: 62.79%; H: 4.68%; N: 0%.

## 2. Molecular weight:

m/e determined by mass spectrum is 344.089 and the theoretical value of m/e for  $\text{C}_{18}\text{H}_{16}\text{O}_7$  is 344.090.

3. Melting point: 190°–192° C.

4. Specific rotation:  $[\alpha]_D^{22} + 32.4^\circ$  (C: 1.02,  $\text{CHCl}_3$ )

5. Ultraviolet absorption spectrum (FIG. 7):

$\lambda_{\text{max}}^{\text{MeOH}}$  nm( $\epsilon$ ): 235 (20700), 265 (12100), 270 (12300), and 342 (3750)

6. Infrared absorption spectrum (FIG. 8):

Relatively strong absorptions at 1765, 1700, 1670  $\text{cm}^{-1}$  when measured by KBr method.

## 7. Solubility:

Soluble in methanol, ethanol, ethylacetate, chloroform, acetone and ether. Insoluble in n-hexane, petroleum ether and water.

## 8. Color reaction:

Positive reaction to reduction catalyst [Feigl, N., Anal. Chem., 28, 397 (1956)]. Negative in ninhydrin reaction, Sakaguchi reaction, Ehrlich reaction, Fehling reaction and Molish reaction.

From the characteristics stated above, it has been found that acetylnanaomycin A is a new compound similar to nanaomycin A.

## (C) Nanaomycin A methyl ester:

## 1. Elementary analysis:

Found: C: 64.84%; H: 5.21%; N: 0%;

Calculated (as  $\text{C}_{17}\text{H}_{16}\text{O}_6$ ): C: 64.55%; H: 5.10%; N: 0%.

## 2. Molecular weight:

m/e determined by mass spectrum is 316.092 and the theoretical value of m/e for  $\text{C}_{17}\text{H}_{16}\text{O}_6$  is 316.090.

## 3. Melting point: 99°–102° C.

## 4. Specific rotation:

$[\alpha]_D^{20} - 12.7^\circ$  (C=1.02,  $\text{CHCl}_3$ )

## 5. Ultraviolet absorption spectrum (FIG. 9):

$\lambda_{\text{max}}^{\text{MeOH}}$  nm( $\epsilon$ ): 248 (12400), 274 (15100), and 424 (5650)

## 6. Infrared absorption spectrum (FIG. 10):

Characteristic and relatively strong absorptions at 1730, 1645 and 1615  $\text{cm}^{-1}$ .

## 7. Solubility:

Soluble in methanol, ethanol, ethylacetate, chloroform, acetone and ether. Insoluble in n-hexane, petroleum ether and water.

## 8. Color Reaction:

Positive reactions to ferric chloride and reduction catalyst [Feigl, N., Anal. Chem., 28, 397 (1956)]. Negative in ninhydrin reaction, Sakaguchi reaction, Ehrlich reaction, Fehling reaction and Molish reaction.

From the characteristics stated above, it has been found that nanaomycin A methyl ester is a new compound similar to nanaomycin A.

The antimicrobial activities of the derivatives of nanaomycin A are shown in Tables 6–8.

TABLE 6

Minimal Inhibitory Concentration of Nanaomycin C		
Test Organisms	Medium	MIC ( $\mu\text{g}/\text{ml}$ )
<i>Staphylococcus aureus</i> FDA 209P	N	6.3
<i>Staphylococcus aureus</i> FDA 209P (JC-1)	N	3.1
<i>Bacillus subtilis</i> PCI 219	N	6.3
<i>Sarcina lutea</i> PCI 1001	N	25
<i>Mycobacterium smegmatis</i> ATCC 607	N	50
<i>Escherichia coli</i> NIHJ	N	100
<i>Escherichia coli</i> NIHJ (JC-2)	N	>100
<i>Klebsiella pneumoniae</i> PCI 602	N	>100
<i>Salmonella typhimurium</i>	N	>100
<i>Shigella flexneri</i>	N	>100
<i>Pseudomonas aeruginosa</i>	N	>100
<i>Candida albicans</i>	P	>100
<i>Saccharomyces sake</i>	P	>100



TABLE 6-continued

Minimal Inhibitory Concentration of Nanaomycin C		
Test Organisms	Medium	MIC (µg/ml)
<i>Aspergillus niger</i>	P	> 100
<i>Trichophyton interdigitale</i>	P	100
<i>Sclerotinia cinerea</i>	P	100
<i>Mycoplasma gallisepticum</i> KP-13	E	12.5
<i>Mycoplasma gallisepticum</i> S-6	E	6.3
<i>Mycoplasma gallisepticum</i> 333P (Spiramycin resistant)	E	3.1
<i>Mycoplasma gallinarum</i>	E	12.5
<i>Mycoplasma iners</i>	E	50
<i>Mycoplasma pneumoniae</i>	E	6.3

Note:  
N - nutrient agar (pH 7.0, 2 days, 37° C.)  
P - potato agar (pH 6.4, 4 days, 27° C.)  
E - Eiken PPLO agar (pH 7.8, 8 days, 37° C.)

TABLE 8

Antimycoplasma Activities of Acetylnanaomycin A and Nanaomycin A Methyl Ester				
Test Organisms	Med.	Inhibition Zone (mm)		
		I	II	A
<i>Mycoplasma gallisepticum</i> KP-13	E	28.5	28.5	28.7
<i>Acholeplasma laidlawii</i> (A)	H	none	none	none

Note:  
E - Eiken PPLO agar (pH 7.8, 8 days, 37° C.)  
H - Hokken PPLO agar (pH 7.8, 8 days, 37° C.)  
I - Acetylnanaomycin A  
II - Nanaomycin A methyl ester  
A - Nanaomycin A

Nanaomycin C inhibits mainly Gram-positive bac-  
15 teria and mycoplasmas, and exerts as strong activity against Gram-positive bacteria as nanaomycin A, but a weaker activity against fungi and mycoplasmas than nanaomycin A.

Acetylnanaomycin A exerts a strong activity against Gram-positive bacteria than nanaomycin A and exerts as strong activity against fungi and mycoplasmas as nanaomycin A. The acute toxicity (LD<sub>50</sub>, intra-pene-

TABLE 7

Minimal Inhibitory Concentrations of Acetylnanaomycin A and Nanaomycin A methyl ester				
Test organisms	Med.	MIC (µg/ml)		Control
		I	II	
<i>Staphylococcus aureus</i> FDA 209P	N	1.6	12.5	3.9
<i>Staphylococcus aureus</i> FDA 209P (JC-1)	N	0.8	25	2.0
<i>Staphylococcus aureus</i> FS 1227 (PC-R)	N	0.8	25	1.6
<i>Staphylococcus aureus</i> KB 61 (R-TC, EM)	N	0.8	12.5	1.6
<i>Staphylococcus aureus</i> KB 64 (R-TC, EM)	N	0.4	12.5	0.8
<i>Bacillus subtilis</i> PCI 219	N	3.1	25	6.3
<i>Bacillus cereus</i> T	N	25	25	12.5
<i>Sarcina lutea</i> PCI 1001	N	1.6	25	1.6
<i>Corynebacterium paurometabolum</i>	N	6.3	25	12.5
<i>Mycobacterium smegmatis</i> ATCC 607	N	50	12.5	100
<i>Aerobacter aerogenes</i> IAM 1183	N	>100	>100	>100
<i>Proteus vulgaris</i> IFO 3167	N	50	>100	50
<i>Proteus mirabilis</i>	N	>100	>100	>100
<i>Escherichia coli</i> NIHJ (JC-2)	N	>100	>100	>100
<i>Salmonella typhimurium</i>	N	50	>100	100
<i>Shigella sonnei</i> E 33	N	100	>100	100
<i>Pseudomonas aeruginosa</i> P-3	N	>100	>100	>100
<i>Candida albicans</i>	P	25	>100	50
<i>Saccharomyces sake</i>	P	12.5	50	12.5
<i>Piricularia orizae</i>	P	0.8	12.5	0.8
<i>Aspergillus niger</i> ATCC 6275	P	25	>100	25
<i>Aspergillus fumigatus</i> IAM 2162	P	6.3	100	6.3
<i>Microsporium gypseum</i> 704	P	0.4	1.6	0.8
<i>Trichophyton asteroides</i>	P	0.8	12.5	0.8
<i>Trichophyton ferrugineum</i>	P	0.8	12.5	0.8
<i>Trichophyton interdigitale</i>	P	1.6	12.5	1.6
<i>Trichophyton mentagrophytes</i>	P	0.4	6.3	<0.2
<i>Trichophyton pedis</i> 804	P	0.8	12.5	0.8
<i>Trichophyton purpureum</i>	P	1.6	12.5	0.4
<i>Trichophyton roseum</i>	P	0.4	0.8	<0.2
<i>Trichophyton rubrum</i>	P	<0.2	<0.2	<0.2
<i>Trichophyton schoenleini</i>	P	<0.2	<0.2	<0.2
<i>Trichophyton violaceum</i>	P	0.8	12.5	3.1

Note:  
N - nutrient agar (pH 7.0, 2 days, 37° C.)  
P - potato agar (pH 6.4, 4 days, 27° C.)  
R - resistant strain  
PC - penicillin  
TC - tetracyclin  
EM - erythromycin  
I - acetylnanaomycin A  
II - nanaomycin A methyl ester  
Control - nanaomycin A



trial injection) in mice of acetylnanaomycin A is 38.5 mg/kg which is lower than that of nanaomycin A.

Antimicrobial activity of nanaomycin A methyl ester is generally lower than those of other derivatives of nanaomycin A.

Therapeutic effect of the derivatives of nanaomycin A of this invention are determined in a similar manner to that applied to nanaomycin A on infectious diseases caused by a parasite of the genus *Tricophyton* in guinea pigs and cattle. Acetylnanaomycin A exhibits superior therapeutic effect to nanaomycin A when its concentration is 0.01–1%. The therapeutic effect of nanaomycin A methyl ester is inferior to those of nanaomycin A and acetylnanaomycin A. Nanaomycin C exhibits a poor therapeutic effect on infectious diseases caused by a parasite of the genus *Trichophyton*.

According to another aspect of the present invention, there is provided a process for producing nanaomycins A and C by fermentation, in which a microorganism

and in a chain of 10 or more. The spores have smooth surfaces.

- 2. Cultural characteristics:  
Shown in Table 9.
- 3. Physiological characteristics:  
Growth temperature: 15°–45° C.  
Liquefaction of gelatin: positive  
Hydrolyzation of starch: positive  
Coagulation of skim milk: positive  
Peptonization of skim milk: positive  
Formation of melanoid pigment: negative  
Formation of tyrosinase: negative  
Reduction of nitrate: positive  
Formation of hydrogen sulfide: negative  
Decomposition of cellulose: negative
- 4. Usability of various carbon sources:  
Arabinose, xylose, glucose, fructose, rhamnose, mannitol, glycerol, maltose and mannose may be usable.  
Sucrose, inositol and raffinose may be unusable.

TABLE 9

Cultural Characteristics of <i>S. rosa</i> var. <i>notoensis</i> (FERM 2209)				
Medium	Growth	Reverse	Aerial mycelium	Soluble pigment
Sucrose-nitrate agar	good, light ivory to light melon yellow	light melon yellow to apricot	light apricot	pearl pink to light melon yellow
Glucose-nitrate agar	good, dusty yellow to golden brown	golden brown to chocolate brown	white to pearl pink	light wheat to sepia brown
Glycerol-asparagine agar	good, light melon yellow to orange rust	apricot	light apricot	melon yellow to apricot
Inorganic salts-starch agar	moderate, light melon yellow	pearl pink to golden brown	white to flesh pink	dark luggage tan to sepia brown
Tyrosine agar	good, light wheat to amber topaz	light mellon yellow to nude tan	light melon yellow to pearl pink	light wheat to melon yellow
Nutrient agar	moderate, colorless to pearl pink	squash yellow to bright yellow	white, scant	none
Glucose-peptone agar	moderate, colorless to golden brown	golden brown to sepia brown	white	ivy to dark laurel
Yeast extract-malt extract agar	good, colorless to golden brown	golden brown to orange rust	light melon yellow to light apricot	ivy
Oatmeal agar	moderate, colorless to light melon yellow	light mellon yellow to nude tan	light melon yellow to light apricot	light tan
Peptone-yeast extract iron agar	moderate, cream to light wheat	colonial yellow	scant, white to colonial yellow	none
Tryptone-yeast extract broth	surface growth, moderate, light ivory	light ivory	white	none
Milk	pearl pink		none	light apricot to pearl pink
Gelatin	surface growth, good	pearl pink to chartreuse tint	white to celadon gray	laurel
Nitrate broth	surface growth, moderate	light ivory	white	none
Cellulose	none	none	none	none

which belongs to the genus *Streptomyces* and which is capable of producing nanaomycins A and/or C is cultured aerobically in a medium conventionally used for fermentation of microorganisms belonging to the genus *Streptomyces* to accumulate nanaomycins A and/or C in the cultured broths and nanaomycins A and/or C accumulated are recovered therefrom.

For the purpose of the present invention, it is possible to use not only the hereinafter described *Streptomyces rosa* var. *notoensis* and any mutant obtained therefrom but also any strain which belongs to the genus *Streptomyces* and which is capable of producing nanaomycins A and/or C. The microbiological characteristics of a preferable strain *Streptomyces rosa* var. *notoensis* which is used in the following examples to produce nanaomycins A and/or C are as follows:

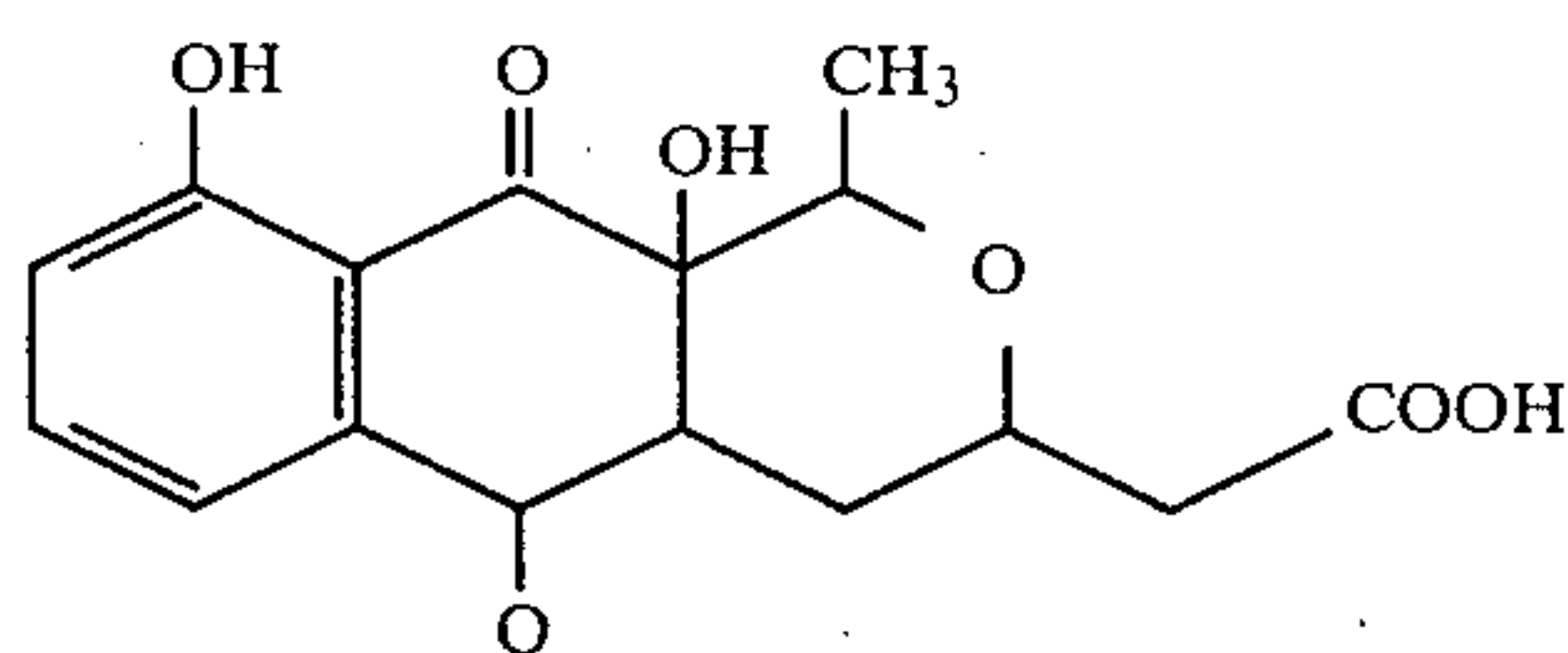
- 1. Morphological characteristics:  
Forming abundantly aerial mycelium on both synthetic and natural agar media, the ending of which forming massy or irregular spiral. Conidiophore formed on aerial mycelium. Conidiospores are oval (0.6–1.0μ)

The microbiological characteristics of this strain are summarized as follows:  
Conidiophore is spiral and conidiospore is smooth. Growth on synthetic medium is colored in yellowish gray or orange gray or reddish brown, and the formed aerial mycelium is colored in white or orange gray or pink. There is formed a soluble pigment colored in yellowish brown or dark reddish brown. When an organic medium is used, the growth is generally colorless or colored in orange gray or brown, and the formed mycelium is colored in white or orange gray or pink. Sometimes soluble pigment is not formed, while a greenish gray or grayish black pigment is formed in some media. This strain is non-chromogenic and has a relatively high activity with regard to the decomposition of protein and starch.  
With respect to the strains having the afore-mentioned characteristics, a search was made for strains having analogous characteristics to those of the strain



used in the following examples with reference, for example, to "The Actinomycetes" by S. A. Waksman, Vol. 2 (1961) and "Cooperative Description of Type Strains of Streptomyces" by E. B. Shirling and D. Gottlieb [International Journal of Systematic Bacteriology, Vol. 18, No. 2, pages 69-189 (1968); Vol. 18, No. 4, pages 279-392 (1969); Vol. 19, No. 4, pages 391-512 (1969); and Vol. 22, No. 4, pages 265-394 (1972)]. As a result, some species designated as "fradiae" i.e. *Streptomyces fradiae*, *Streptomyces luridus*, *Streptomyces roseus*, *Streptomyces fuscus*, *Streptomyces roseoluteus*, and *Streptomyces rosa* were found as being analogous. Among them, *Streptomyces roseoluteus* and *Streptomyces rosa* are indeed likely to be most analogous. However, on one hand *Streptomyces roseoluteus* is distinguishable from the nanaomycin-producing strain of the present invention because the color at the reverse side of *S. roseoluteus*'s colony becomes yellowish orange from yellow in certain media such as for example of yeast extract-malt extract, oat meal agar, inorganic starch agar as well as of glycerol-asparagine agar, with simultaneously formation of yellowish soluble pigment. On the other hand, *Streptomyces rosa* is generally similar to the nanaomycin-producing strain according to the present invention with the exception that the production of soluble pigment in certain media such as for example of yeast extract-malt extract agar, glucose-peptone agar and that the reduction of nitrate is not observed in the case of *Streptomyces rosa*. Accordingly, this strain is designated as *Streptomyces rosa* var. *notoensis*.

The nanaomycin-producing strain used in the following examples produces simultaneously in the cultured broths nanaomycins A and C and nanaomycin B represented by general formula:



and referred to in the related U.S. patent application entitled "New Compound Nanaomycin B and A Process for Producing the Same by Fermentation" and filed concurrently with this application.

The strain used in the following examples has been deposited on an unrestricted basis with the Fermentation Research Institute, Agency of Industrial Science and Technology, Japanese Government and assigned an accession number of FERM-P No. 2209.

According to the process of the present invention, either any synthetic or organic medium may be used when it contains a suitable carbon source, nitrogen source, inorganic substances and, if desired, various other nutrients. Various carbon and nitrogen sources may be used when these sources are adaptable for the strain in use.

More concretely, the useful carbon sources are exemplified by various carbohydrates such as glucose, glycerol, fructose, maltose, mannitol, xylose, galactose, lactose, ribose, starch and starch hydrolyzate. The concentration of carbon source is preferably 0.5-5.0% (when calculated as glucose) based upon the medium. It is also possible to use organic acids such as, for example, gluconic acid, pyruvic acid, lactic acid, acetic acid; and

various amino acids such as glycine, glutamic acid, alanine etc.

As the nitrogen source, it is possible to use, for example, ammonia; various inorganic and organic ammonium salts such as ammonium chloride, ammonium phosphate, ammonium sulfate, ammonium nitrate; nitrogen-containing organic materials such as urea, peptone, NZ-amine, meat extract, dried yeast, yeast extract, corn steep liquor, casein hydrolyzate, fish meal, digested product thereof, soybean meal, digested products thereof, defatted soybean, digested products thereof, pupa hydrolyzate, and various amino acids such as glycine, glutamic acid, alanine etc.

As the inorganic substance, it is possible to use, for example, various phosphates, magnesium sulfate etc. If desired, it is also possible to use a trace amount of heavy metal salts, which is, however, not always essential when the medium used contains natural materials. In case a mutant strain having a nutritional requirement is used, it is necessary to add the required substance to the medium.

Liquid medium is preferable for producing large amount of nanaomycins A, B and C though solid medium may be used. It is possible to use a seed medium having a similar composition to that of the main culture medium, and the seed is preferably obtained by fermentation carried out aerobically at a temperature of 27° C. for two days, for example, by using a Sakaguchi flask.

The fermentation is carried out aerobically with shaking and/or submerged conditions at a temperature of from 15° to 40° C. at an adjusted pH of 6-10 for about 2-8 days, whereby large amounts of nanaomycins A, B and C are accumulated concurrently in the medium and microbial body. After completion of the fermentation, nanaomycins A, B and C are recovered from the cultured broths. For example, the broths are separated into the microbial body and filtrate. The filtrate is adjusted to an acidic pH (preferably from 2 to 4) with HCl or the like and is then subjected to extraction with a suitable organic solvent such as e.g. ethyl acetate or butyl acetate. After this, nanaomycins A, B and C are obtained by purifying the extracted substance in a conventional manner which would be used for the purification of known substances soluble in organic solvents.

According to the present invention, preferable methods for the fermentation are exemplified as follows:

A culture medium (100 ml) is put in a 500 ml Sakaguchi flask and sterilized at a temperature of 120° C. for 15 minutes. After this, spores and/or mycelium of the used strain are inoculated and the fermentation is effected with shaking (110 r.p.m.) at a temperature of 27° C. for a sufficient period of time (e.g. for 3 days) to accumulate large amounts of nanaomycins A, B and C in the culture broths.

Alternatively, a culture medium (20 liters) is put into a 30-liter jar fermentor and sterilized at a temperature of 120° C. for 15 minutes. After this, a seed culture is inoculated and the fermentation is effected at a temperature of 27° C. for 3 days with shaking (300 r.p.m.) and aeration (10 l/min). It is also preferred to culture using a medium (200 ml) in a tank-type fermentor (capacity-400 liters) at a temperature of 27° C. for 3 days with shaking (200 r.p.m.) and aeration (100 l/min).

In either case, good results can be obtained by using glycerine and soybean meal as the carbon and nitrogen sources, respectively. A medium containing glycerine (2.0%) soybean meal (2.0%) and NaCl (0.3%) and having a pH of 7.0 is particularly advantageous. In one



embodiment using this medium, an inhibition zone (diameter—30 cm) is observed in the supernatant of the cultured liquor at a pH of 5.2 after culturing at a temperature of 27° C. for 70 hours by using a tank-type fermentor. Although nanaomycins A, B and C are found in both the fermented liquid and solid materials, the former contains usually larger amounts of nanaomycins A, B and C than the latter.

After completion of the fermentation, nanaomycins A and C are recovered from the cultured broths in the following manner:

The cultured broths are separated into solid and liquid phases in conventional manner by means of filtering, centrifuging and the like. The liquid phase, i.e. the filtrate is adjusted to an acidic pH (preferably from 2 to 4) with HCl or the like and is then subjected to extraction with a suitable organic solvent such as e.g. ethyl acetate or butyl acetate. After this, nanaomycins are obtained by purifying the extracted substance in a conventional manner which is applicable for the purification of known substances soluble in organic solvents.

It is also possible to isolate nanaomycins from the extracted solution in an acidic condition. For example, an aqueous solution of sodium bicarbonate (1%) is used to elute nanaomycins A and B from the extract with quick speed. Immediately after this, the eluate containing nanaomycins A and B is adjusted to an acidic pH, for example, with hydrochloric acid, and further extracted with a suitable organic solvent such as, for example, ethyl acetate or butyl acetate. The thus-obtained extract is concentrated to dryness, resulting in nanaomycins A and B in the form of crude powders which are then subjected to column chromatography using silica gel, whereby the crude powders containing nanaomycins A and B are developed with a solvent system of benzene-acetone (4:1 v/v) to elute the fractions containing nanaomycin A followed by nanaomycin B-containing fractions. The thus-obtained fractions are separately combined and concentrated to dryness. The dried material containing nanaomycin A is dissolved in ethanol which is then added with a small amount of water to give nanaomycin A in the form of needle crystals. Nanaomycin B can also be purified in a similar manner to that applied to nanaomycin A.

In the recovery, nanaomycins A and B are eluted from the ethyl acetate layer with 1% of sodium bicarbonate, while nanaomycin C remains in the layer because nanaomycin C is neutral, which is then concentrated under reduced pressure to dryness to obtain the crude powders of nanaomycin C. The crude powders are chromatographed on a column of silica gel with chloroform-methanol (50:1 v/v). The fractions containing nanaomycin C are concentrated under reduced pressure to dryness to obtain crude powders of nanaomycin C which are extracted with ethyl acetate and then recrystallized from an ethyl acetate to obtain orange crystals of nanaomycin C.

Nanaomycin A is also obtained from nanaomycin B (hereinbefore referred to) in an alkaline medium in the following manner:

Nanaomycin B (200 mg) is dissolved in 60 ml of 0.1 N sodium hydroxide and the solution is allowed to stand for 10 minutes. After adjusting to pH 2.0 with 6 N hydrochloric acid, the product is extracted with ethyl acetate. The extract is evaporated and orange yellow needles are obtained from an ethanol solution of the product. The compound is identified as nanaomycin A

by the melting point, IR spectrum and thin-layer chromatography.

According to the present invention, acetylnanaomycin A and nanaomycin A methyl ester can be obtained in the following manner:

Nanaomycin A is left in a solution containing acetic anhydride and pyridine, poured onto a mixture of ice and hydrochloric acid, and is then extracted with chloroform. The extract is washed with water, dried, and then recrystallized from benzene to obtain acetylnanaomycin A in the form of crystals of pale yellow needles.

Nanaomycin A is dissolved in ether and treated with an ethereal solution of diazomethane. After removal of the solvent a column chromatography on silica gel with a chloroform-methanol system gives orange-yellow nanaomycin A methyl ester.

Nanaomycin A and derivatives thereof according to the present invention are assayed in a similar manner to that designated as the paper disc method by Itoh, et al [J. of Antibiotics, 24, 855-859 (1971)], for example, as follows:

The strain is cultured at a temperature of 27° C. with shaking. The medium (pH 7.0) contains glycerine (2.0%), soybean meal (2.0%) and NaCl (0.3%). Inhibition zones of a diameter of 17, 27, 28 and 29 mm are observed in the cultured liquor after culturing for 24, 48, 72 and 96 hours, respectively.

The following non-limitative examples illustrate the invention.

#### EXAMPLE 1

One platinum loop of *Streptomyces rosa* var. *notoensis* FERM-P No. 2209 capable of producing nanaomycins was taken from a slant culture and inoculated to a seed medium (pH 7.0) for culturing at a temperature of 27° C. for 2 days. The resultant seed culture was further inoculated to a medium (20 liters) put in a 30-liter jar fermentor at a ratio of 1% and cultured at 27° C. for 4 days with aeration (10 l/min) and agitation (300 r.p.m.). These media contained 2.0% of glycerol, 2.0% of soybean meal and 0.3% of NaCl and had an adjusted pH of 7.0. The media were sterilized at a temperature of 120° C. for 15 minutes before use. After completion of the fermentation, the pH of the cultured broths was 4.8 and an inhibition zone against *Mycoplasma gallisepticum* (diameter-30 mm) was observed in the supernatant of the broths. The broths (20 liters) were subjected to centrifugation to remove the mycelium. The filtrate was adjusted to a pH of 2.0 with 6 N HCl and was then subjected to extraction with butyl acetate (4 liters). The butyl acetate layer was extracted with 1% sodium bicarbonate aqueous solution (800 ml). The aqueous layer was adjusted to a pH of 2.0 with 6 N HCl and was subjected to extraction with ethyl acetate. The ethyl acetate layer was concentrated and added with petroleum ether to give yellow-brown powders (1.09 g) which were further purified in the following manner.

The crude powders containing nanaomycins A and B were dissolved in ethyl acetate (15 ml), added with silica-gel (4 g) and then concentrated in vacuo to dryness. The dried material was transferred to a column packed with silica gel (55 g) and developed with a solvent system of benzene-ethyl acetate (4:1 v/v). The eluate was divided into individual fractions (each 15 ml). Each fraction was then assayed by the above-described paper disc method using *Mycoplasma gallisepticum* KP-13 as a test microorganism. The first part of the eluate, i.e., Nos. 8 to 22 of the fractions contained



nanaomycin A, and No. 14 exhibited a highest activity against the test microorganism. For fractions after No. 30, another solvent system of benzene-ethyl acetate (3:1 v/v) was used as the eluting solution. Nos. 32 to 60 of the divided fractions contained nanaomycin B, and the activity of No. 46 was highest against the control microorganism. The fractions Nos. 8 to 22 were combined and concentrated in vacuo to dryness. The dried solid material was dissolved in ethanol and then added with a small amount of water to give yellow needle crystals (31.7 mg). The crystals were recrystallized from an ethanol solution in a similar manner to that described above to give a purified nanaomycin A (25.3 mg; purity: more than 99%; melting point: 178°–180° C.).

UV absorption spectrum of nanaomycin A:

$\lambda_{max}^{Me-OH}nm$ : 250, 274 and 423

IR absorption spectrum of nanaomycin A:

Characteristic strong absorptions at 1725, 1640 and 1610  $cm^{-1}$  when measured by KBr method.

The second part of the fractions (Nos. 32 to 60) was combined and concentrated in vacuo to dryness to give pale yellow powders (450 mg). The powders were further subjected to column chromatography using silica gel in a similar manner to that described above to obtain purified powders of nanaomycin B (270 mg; purity: 99%; melting point: 84°–86° C.).

UV absorption spectrum of nanaomycin B:

$\lambda_{max}^{MeOH}nm$ : 231 and 352

IR absorption spectrum of nanaomycin B:

Characteristic strong absorptions at 1705, 1648 and 1605  $cm^{-1}$  when measured by KBr method.

When the butyl acetate layer was extracted with 1% sodium bicarbonate solution as stated above, nanaomycin C was retained in the butyl acetate layer owing to nanaomycin C being neutral. The solvent layer was concentrated and dried to give crude powders (1.3 g) of nanaomycin C which was then chromatographed on a column of silica gel with chloroform-methanol (50:1 v/v). The obtained fractions (each 15 ml) were tested by the above-described paper disc method. Among the total 58 fractions, the fractions of Nos. 51–63 contained nanaomycin C, and the No. 55 fraction exhibited a highest activity against the test microorganism. The fractions containing nanaomycin C were combined and concentrated in vacuo to dryness. The thus obtained solid material (52 mg) was extracted with ethyl acetate and recrystallized from ethyl acetate to give orange-yellow nanaomycin C (35 mg) in the form of needle crystals. Purity: 99% Melting point: 222°–224° C.

UV absorption spectrum of nanaomycin C:

$\lambda_{max}^{MeOH}nm(\epsilon)$ : 248 (10100), 274 (12400) and 424 (4610).

IR absorption spectrum:

Characteristic absorptions at 1645 and 1605  $cm^{-1}$ .

#### EXAMPLE 2

To the solid material which was obtained by centrifugation of the cultured broths prepared in a similar manner to that described in Example 1, there was added ethyl acetate (5 liters) with agitation. The thus-obtained extract was added with 1% solution of sodium bicarbonate (2 liters) to transfer the material including nanaomycins A and B to the aqueous layer. The aqueous layer was adjusted to a pH of 2.0 with hydrochloric acid and was then subjected to extraction with ethyl acetate (500 ml). The extracted solution was concentrated in vacuo to dryness to give crude powders (521 mg) in yellow brown. The powders were subjected to silica gel col-

umn chromatography and the eluate was treated to obtain nanaomycins A and B in a similar manner to that described in Example 1. By extracting with 1% sodium bicarbonate stated above, nanaomycin C-containing ethyl acetate layer was concentrated to dryness to obtain crude powders of nanaomycin C (685 mg). The powders were treated in a similar manner to that described in Example 1 to give a solid material (28.3 mg) from which nanaomycin C was obtained.

	Yield	Melting Point	Purity
Nanaomycin A	13 mg	173–175° C.	95%
Nanaomycin B	85 mg	82–84° C.	92%
Nanaomycin C	19.7 mg	220–222° C.	98%

#### EXAMPLE 3

Nanaomycin A (200 mg), which was obtained in a similar manner to that described in Example 1, was left in a solution containing acetic anhydride (2 ml) and pyridine (4 ml) for 16 hours at room temperature. The solution was then poured onto a mixture of ice water and 10% hydrochloric acid and extracted with chloroform (30 ml). The extract was washed with water and concentrated in vacuo to dryness. The thus obtained solid material was recrystallized from benzene to give pale yellow acetylnanaomycin A (145 mg) in the form of needle crystals. Purity—more than 99%; Melting point: 190°–192° C.

UV absorption spectrum:

$\lambda_{max}^{MeOH}nm(\epsilon)$ : 235 (20700), 265 (12100), 270 (12300) and 342 (3750).

IR absorption spectrum (by KBr method):

Characteristic absorptions at 1765, 1700 and 1670  $cm^{-1}$ .

#### EXAMPLE 4

Nanaomycin A (200 mg), which was obtained in similar manner to that described in Example 1, was dissolved in ether (30 ml), added with an excess of an ethereal solution of diazomethane and left for one hour at room temperature. After removing the solvent in vacuo, the reaction material was chromatographed on a column of silica-gel (6 g) with chloroform-methanol (100:1 v/v). The obtained fractions (each 5 ml) were tested by the above-described paper disc method. The fractions of Nos. 5–9 contained nanaomycin A methyl ester, and No. 6 fraction exhibited a highest activity against the test microorganism. The fractions containing nanaomycin A methyl ester were concentrated and dried to obtain orange-yellow powders of nanaomycin A methyl ester (34 mg). Purity: more than 97%. Melting point: 99°–102° C.

UV absorption spectrum:

$\lambda_{max}^{MeOH}nm(\epsilon)$ : 248 (12400), 274 (15100), and 424 (5650).

IR absorption spectrum (by KBr method):

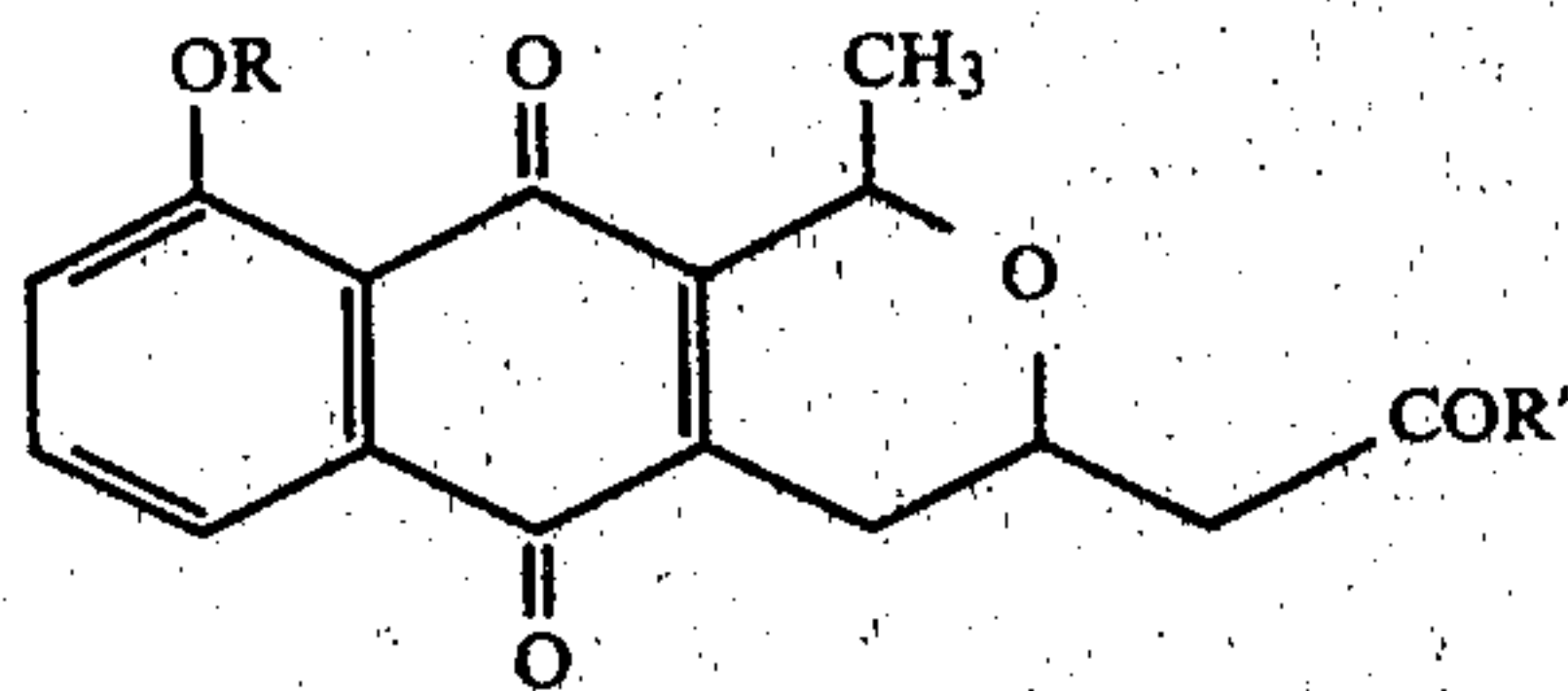
Characteristic absorptions at 1730, 1645 and 1615  $cm^{-1}$ .

What is claimed is:

1. New compounds represented by the following general formula:



17



wherein

(a)  $R=H$ ,  $R'=OH$  and the compound has a specific rotation

$[\alpha]_D^{26} -27.5^\circ$  ( $C=1.0$  in methanol)

(b)  $R=H$ ,  $R'=NH_2$  and the compound has a specific rotation

$[\alpha]_D^{26} -2^\circ$  ( $C=0.5$  in dioxane)

18

(c)  $R=COCH_3$ ,  $R'=OH$  and the compound has a specific rotation  $[\alpha]_D^{22} +32.4^\circ$  ( $C=1.02$  in  $CHCl_3$ )

(d)  $R=H$ ,  $R'=OCH_3$  and the compound has a specific rotation

5  $[\alpha]_D^{20} -21.7^\circ$  ( $C=1.02$  in  $CHCl_3$ )

2. The compound of claim 1 wherein  $R=H$  and  $R'=OH$ , said compound being denoted Nanaomycin A.

3. The compound of claim 1 wherein  $R=H$  and  $R'=NH_2$ , said compound being denoted Nanaomycin

10 C.

4. The compound of claim 1 wherein  $R=COCH_3$  and  $R'=OH$ , said compound being denoted acetyl Nanaomycin A.

5. The compound of claim 1 wherein  $R=H$  and  $R'=OCH_3$ , said compound being denoted Nanaomycin A methyl ester.

\* \* \* \* \*

20

25

30

35

40

45

50

55

60

65